Oxygen Microscopy with Two-photon-enhanced Phosphorescent Nanoprobes

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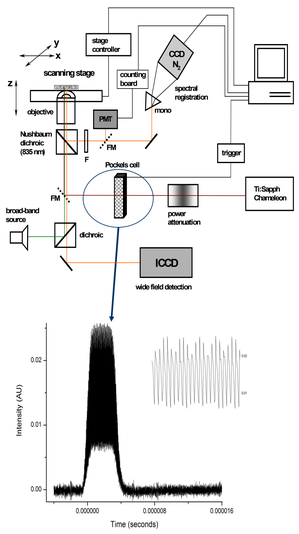
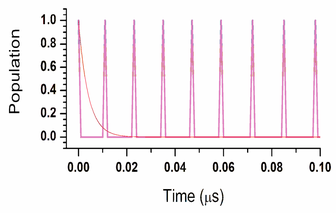
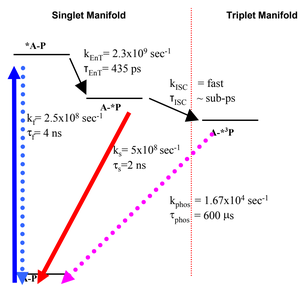
Abstract

Cellular or tissue pO_2 can be measured optically by the phosphorescence quenching method [1] using probes with controllable quenching parameters and defined bio-distributions [2]. We have described a new approach to oxygen imaging by phosphorescence, which draws from two-photon laser scanning microscopy (2P LSM) [3]. Intuitively, coupling phosphorescence imaging with 2P LSM appears to be a straightforward task. However, several critical issues must be dealt with: (i) design of probe molecules with the required two-photon absorption (2PA) cross-sections (σ^2), high quantum yield of phosphorescence, and appropriate biological partitioning; (ii) development of code and methods to integrate measurement of long-lived species (microsecond to millisecond) with scanning stage microscopy; (iii) validation of these new probes and methods compared to traditional fluorescence based TPA systems. We are also developing methods to deliver the probes to the cellular environment. The most promising method for probe delivery is a nano-carrier called a polymersome; similar to a liposome, but made of polymers. Our progress in these areas will be reported on.

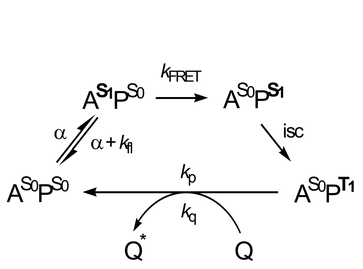
Two-photon Phosphorescence Lifetime Microscopy (2PLM)

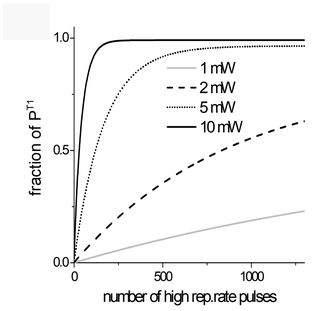


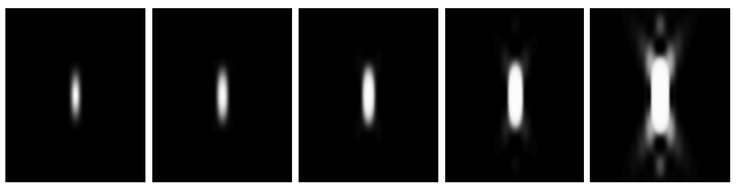




Resolution vs. Signal







P=2 mW	P=5 mW	P=5 mW	P=10 mW	P=20 mW
δt=2 μs	δt=2 μs	δt=5 μs	δt=5 μs	δt=10 μs
S=1.0	S=4.9	S=9.3	S=22.6	S=86.0

References

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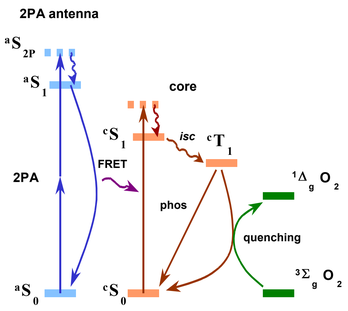
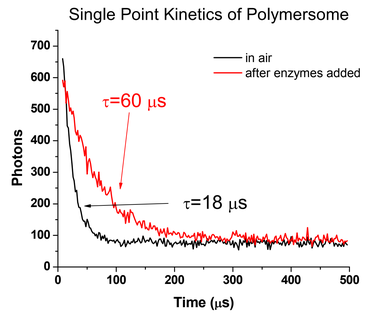
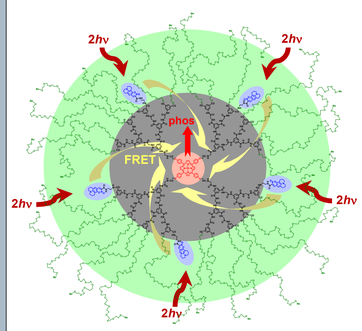
Oxygen Imaging Via Phosphorescence Quenching



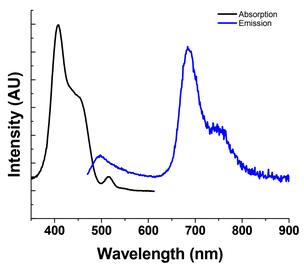


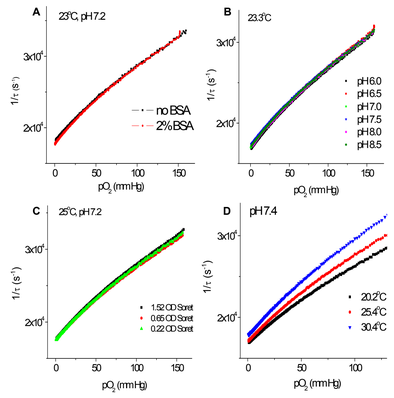
Probe Design



- Antenna (coumarins) has high two photon absorption cross-section
- Dendrimer modulates sensitivity to oxygen and protects from other quenches
- PEGs provide water solubility and deters aggregation
- Pd or Pt porphyrin core senses oxygen

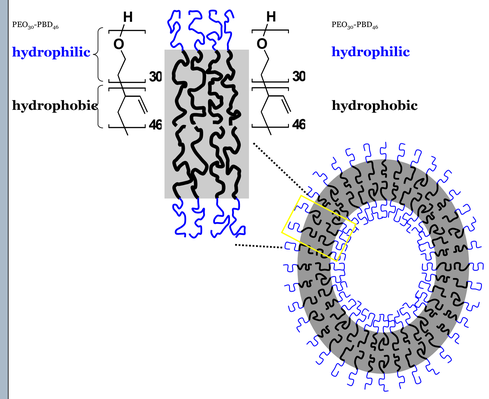
Additional probe details found in ref. [4]





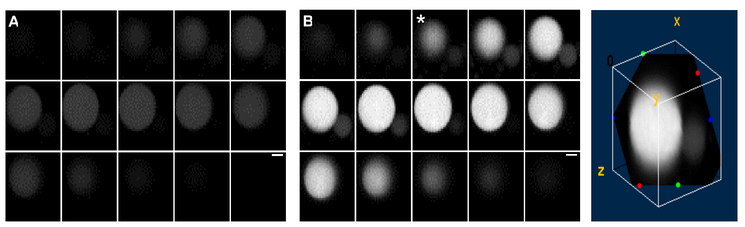
Oxygen Quenching under various conditions: A) Presence and absence of protein. B) pH. C) concentrations probe. D) temperature

Polymersomes



- 5-50 times tougher than liposomes
- Potential incorporation of large hydrophobic molecules
- **Potential for variety of modifications**
- Diversity of membrane polymers
- Potential for membrane functionalization
- Capable of biological activity
- PEO: FDA approved homopolymer that imparts to vesicles surface biocompatability and prolonged blood circulation times

Additional polymersome details in ref. [5]

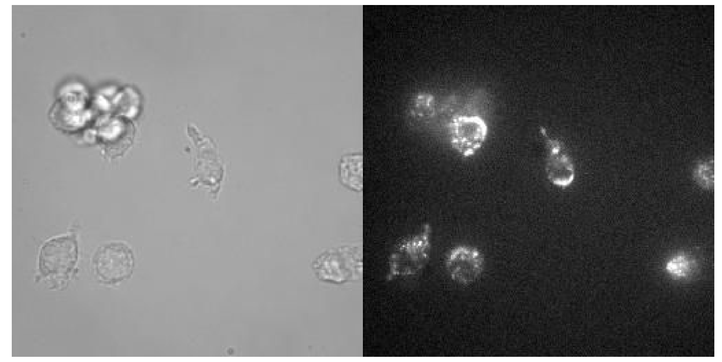


Z-scan of a polymersome attached to the glass surface, p=14.4 mW. Image shows phosphorescent photons after removal of the scatter and the background. XY cross-sections are taken in 2 mm steps in Z from the bottom up (left-to-right, top-to-bottom in the images). A) Polymersomes in a cuvette equilibrated with air. B) The same sample imaged after addition of glucose oxidase/catalase enzymatic system to deoxygenate the solution. Scale bar - 5 mm. [6]

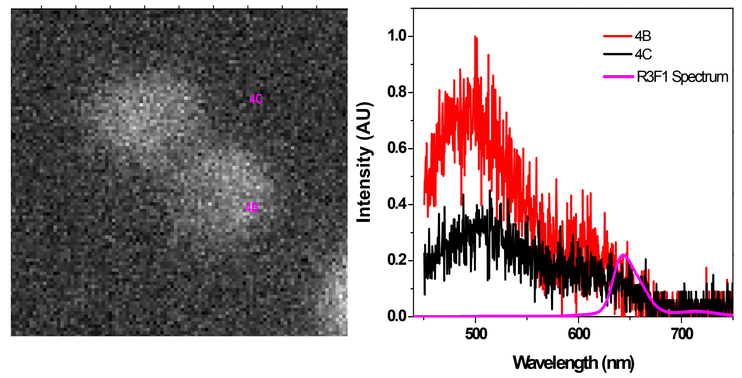
Delivery in to Cells

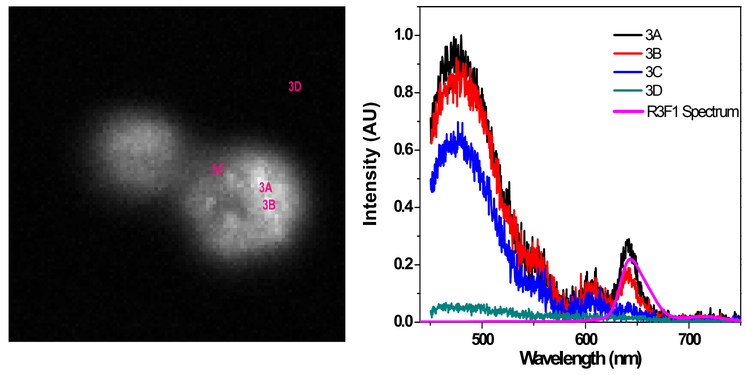
TAT-functionalized polymersomes were formed using the thin-film hydration method; the hydrating solution contained 100 μM of the fluorescent probe R3F1 (analog of oxygen probe). This is a fluorescent analog of our oxygen sensing probe, which can easily be monitored via standard microcopy techniques. Dynamic light scattering indicated the average polymersome diameter was 186 nm. The dye-loading polymersome was introduced to RAW 264.7 macrophages (murine), and incubated overnight. The samples were fixed with 4% PFA.

Widefield emission spectroscopy showed that emission was co-localized with the cells. The bright pixels in the dye exposed plates were ~3-10x brighter than the brightest pixels in the control plates. The dye exposed plates each had several thousand pixels with intensity above 5,000, while the control plates had less than 50. To confirm the emission was from R3F1, we performed spectral imaging. All bright pixels examined in the dye-loaded plates had emission spectra corresponding to R3F1, while the control plates showed emission spectra that is attributed to auto-fluorescence.



Left: Transmitted light image of cells loaded with R3F1. Right: Fluorescence image of same cells showing emission arises from the cells.





Top: A control cell (left) and spectra taken from the points noted (right). Spectra only show auto-fluorescence. Bottom: Cell loaded with R3F1 (left) and spectra taken from points noted (right). Spectra taken from pixels inside cell have the characteristic R3F1 spectra signature, in addition to auto-fluorescence.

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